R E M A R K S

Reconsideration of this application, is respectfully requested.

THE PRIOR ART REJECTION

Submitted herewith is an accurate English translation of the priority document of the present application, namely Japanese Patent Application No. 2000-333782, filed in Japan on October 31, 2000, the priority of which is claimed under 35 USC 119. As clearly seen from the translation of Japanese priority application No. 2000-333782, the claims in the present application are fully supported by the disclosure of said Japanese priority application No. 2000-333782 and are entitled to the priority date of October 31, 2000 (which is earlier than the U.S. filing date of the Birk et al reference).

In view of the submission of the accurate English translation of Japanese priority application No. 2000-333782, which predates the U.S. filing date of Birk et al (USP 6,611,643), it is respectfully submitted that Birk et al. is not a proper reference against the present application, and all of the rejections based on Birk et al, as set forth in Office Action dated April 7, 2004, are no longer applicable.

* *

In view of the foregoing, it is respectfully submitted that all of the pending claims are now in condition for immediate allowance.

Accordingly, entry of this Response, allowance of the claims and the passing of this application to issue are respectfully solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,

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TRANSLATION

I, Yuko Mitsui, residing at 4-6-10, Higashikoigakubo, Kokubunji-shi, Tokyo, Japan, state:

that I know well both the Japanese and English languages,

that I translated, from Japanese into English, Japanese Patent Application No. 2000-333782, filed on October 31, 2000, and

that the attached English translation is a true and accurate translation to the best of my knowledge and belief.

Dated: June 28, 2004

Yuko Mitsui

Yuko Mitsui

[Name of Document]	PATENT APPLICATION
[Reference Number]	A000006337
[Filing Date]	October 31, 2000
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[Title of the Invention]	LASER MICROSCOPE
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This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: October 31, 2000

Application Number: Patent Application No. 2000-333782

Applicant(s): OLYMPUS OPTICAL CO., LTD.

This 3rd day of October 2001

Commissioner,
Patent Office

Kozo OIKAWA (seal)

Certificate No.2001-3090432

[Document] SPECIFICATION

[Title of the Invention] LASER MICROSCOPE

[What is Claimed is:]

[Claim 1] A laser microscope, which irradiates a sample with a laser light constituted of a plurality of emission wavelengths through an objective lens, and detects a fluorescent light from the sample, the laser microscope characterized by comprising:

spectral resolution means for spectrally resolving the laser light constituted of a plurality of emission wavelengths;

a light receiving element array configured to receive the laser lights spectrally resolved by the spectral resolution means; and

control means for receiving an output signal of the light receiving element array and controlling the laser light for each of the emission wavelengths.

[Claim 2] The laser microscope according to claim 1, characterized in that the spectral resolution means is any one selected from a group including a prism, a diffraction grating, and a beam splitter.

[Claim 3] The laser microscope according to claim 1 or 2, characterized in that the light receiving element array comprises either one of a split photodiode and a solid image pickup element.

[Claim 4] The laser microscope according to claim 1, 2 or 3, characterized by comprising an optical fiber for

guiding the laser light constituted of a plurality of emission wavelengths into a laser microscope main body.

[Claim 5] The laser microscope according to claim 4, characterized by comprising:

a collimator lens configured to collimate the laser light; a beam splitter configured to split a part of the laser light collimated by the collimator lens; spectral resolution means for spectrally resolving the laser light split by the beam splitter; a converging lens configured to converge the laser light spectrally resolved by the spectral resolution means; and a light receiving element array configured to receive the laser lights converged by the converging lens, those of which being formed into one block constituted to be attachable/detachable with respect to a main body of the laser microscope.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention relates to a laser microscope for use particularly in biological, medical, and other applications, which irradiates a sample with a laser light constituted of a plurality of emission wavelengths through an objective lens and detects fluorescent light from the sample.

[0002]

[Prior Art]

There is a laser microscope for use in biological, medical, and other applications. In the laser microscope,

for example, it is requested to observe a live cell or tissue over a long time as it is. For example, there is an observation of a change of a concentration of calcium in the cell or the tissue. A method of the observation comprises: dyeing a sample with a fluorescent indicator which emits a fluorescent light in accordance with the calcium concentration; irradiating the sample with a laser light (excitation light) of a wavelength suitable for the fluorescent indicator; and detecting the fluorescent light from the sample. In this case, the change of a signal (fluorescent light) from the cell or the tissue is generally remarkably small. Therefore, an intensity of the laser light with which the sample is irradiated is required to be stable at a high precision over a long time.

[0003]

Some causes for which the intensity of the laser light for the irradiation of the sample is not stable are considered. Control of the intensity of the laser light, which includes monitoring and feeding the emission output is not performed. Therefore, the emission output of the helium neon laser fluctuates with a change of environmental temperature, and the like.

[0004]

Moreover, there is a multi-wavelength oscillation. For example, argon lasers oscillate with the laser light of wavelengths of 488 nm, 514.5 nm. Some of the argon lasers monitor and feedback-control the emission output. However,

the argon laser monitors a general output of the argon laser light of the wavelengths of 488 nm, 514.5 nm. The outputs of respective lines of these wavelengths compete among emission modes (wavelengths of 488 nm, 514.5 nm), and thereby each emission wavelength fluctuates. Furthermore, by consumption of an argon gas, an intensity ratio of the emission outputs of the argon laser light (intensity ratio of the wavelengths of 488 nm, 514.5 nm) changes with a use time.

[0005]

On the other hand, there is a laser microscope for introducing the laser light into an optical fiber and guiding the laser light into a laser microscope main body by the optical fiber. In the laser microscope, the intensity of the laser light with which the sample is irradiated fluctuates by an output fluctuation by the optical fiber during undergoing of the change of environmental temperature, and a fluctuation of a light introduction efficiency by thermal deformation of a constituting element.

[0006]

If the intensity of the laser light fluctuates by the aforementioned causes, although the signal (fluorescent light) from the sample does not actually change, an erroneous result is possibly caused as if there were the change of the signal.

[0007]

A technique for stabilizing the intensity of the laser light with which the sample is irradiated is disclosed, for

example, in Jpn. Pat. Appln. KOKAI Publication Nos. 11-231222 and 2000-206415. In the Jpn. Pat. Appln. KOKAI Publication No. 11-231222, after the laser lights of a plurality of wavelengths are combined, some of the laser lights are split by a beam splitter. Subsequently, a changeable filter selects the wavelength, and an optical detector (first detection element) receives the laser light of the selected wavelength, and detects the intensity of the laser light of the wavelength. Moreover, a laser output or the laser intensity is controlled based on a detection signal of the laser light intensity. It is described that the laser intensity is controlled, for example, by an acousto-optical element (e.g., AOTF) disposed between the laser and the optical fiber.

[0008]

The Jpn. Pat. Appln. KOKAI Publication No. 2000-206415 discloses a method comprising: controlling an operation in combination with a linear filter ring driven by a control unit, an area selection filter ring, or a filter slider; detecting an output of a selected laser line; driving the AOTF based on the detection signal; and stabilizing the output of the selected laser line, in order to constantly monitor laser radiation connected to a scanning module.

[0009]

[Object of the Invention]

In recent years, in order to further pursue a function of the cell or the tissue, it has strongly been requested to

simultaneously detect two or more types of signal (fluorescent light) from the sample, and analyze the function. For example, for fluorescent proteins of different wavelengths, such as a green fluorescent protein (GFP: a protein emitting a green fluorescent light) and a red fluorescent protein (RFP: a protein emitting a red fluorescent light), a gene is developed in the cell, and observed with time.

[0010]

In this case, the laser light with which the sample is to be irradiated needs to have a wavelength optimum for these fluorescent proteins GFP, RFP. Additionally, both the laser lights of the two wavelengths need to have the light intensities stabilized.

[0011]

However, in the techniques described in the two publications, only the intensity of the laser light of one wavelength is stabilized, and the laser lights of two or more wavelengths cannot simultaneously be controlled so as to stabilize the intensities of the laser lights.

[0012]

To solve the problem, an object of the present invention is to provide a laser microscope capable of simultaneously and steadily controlling an intensity of a laser light constituted of a plurality of wavelengths with which a sample is to be irradiated for each wavelength.

[0013]

[Means for Achieving the Object]

According to the present invention of claim 1, there is provided a laser microscope, which irradiate a sample with a laser light constituted of a plurality of emission wavelengths through an objective lens, and detecting a fluorescent light from the sample the laser microscope characterized by comprising: a spectral resolution means configured to spectrally resolve the laser light constituted of a plurality of emission wavelengths; a light receiving element array configured to receive the laser lights spectrally resolved by the spectral resolution means; and a control means configured to receive an output signal of the light receiving element array and controlling the laser light for each of the emission wavelengths.

[0014]

According to the present invention of claim 2, there is provided the laser microscope according to claim 1, characterized in that the spectral resolution means is any one selected from a group including of a prism, a diffraction grating, and a beam splitter.

[0015]

According to the present invention of claim 3, there is provided the laser microscope according to claim 1 or 2, characterized in that the light receiving element array comprises either one of a split photodiode and a solid image pickup element.

[0016]

According to the present invention of claim 4, there is provided the laser microscope according to claim 1, 2 or 3, characterized by comprising an optical fiber for guiding the laser light constituted of a plurality of emission wavelengths into a laser microscope main body.

[0017]

According to the present invention of claim 5, there is provided the laser microscope according to claim 4, characterized by comprising: a collimator lens configured to collimate the laser light; a beam splitter configured to split a part of the laser light collimated by the collimator lens; a spectral resolution means configured to spectrally resolve the laser light split by the beam splitter; a converging lens configured to converge the laser light spectrally resolved by the spectral resolution means; and a light receiving element array configured to receive the laser lights converged by the converging lens, those of which being formed into one block constituted to be attachable/ detachable with respect to a main body of the laser microscope.

[0018]

[Embodiments of the Invention]

(1) A first embodiment of the present invention will be described hereinafter with reference to the accompanying drawings.

[0019]

FIG. 1 is a constitution diagram of a laser scanning microscope. An argon laser 2 is fixed on a base 1. The argon laser 2 emits a laser light of two wavelengths of $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm.

[0020]

A scanning unit 3 constituting the laser microscope main body is disposed on an optical path of the laser light emitted from the argon laser 2. The scanning unit 3 is a unit which scans the laser light on a sample 4. Therefore, in this scanning unit 3, a beam splitter 5 is disposed on the optical path of the laser light, and an X-Y scanner 6 is disposed on a reflection optical path of the beam splitter 5.

[0021]

The sample 4 is a cell in which, for example, a GFP and a yellow fluorescent protein (YFP) are subjected to gene development.

[0022]

Moreover, a dichroic mirror 8 and mirror 9 are disposed in series via a mirror 7 on a transmission optical path (optical path of a direction in which the light is incident upon the beam splitter 5 from the X-Y scanner 6) of the beam splitter 5. The dichroic mirror 8 splits a fluorescent light of two wavelengths $\lambda 1'$, $\lambda 2'$ emitted when the sample 4 is irradiated with the laser light of two wavelengths of 488 nm and 514.5 nm. That is, the dichroic mirror 8 has a function for reflecting the fluorescent light of one wavelength $\lambda 1'$

and transmitting the fluorescent light of the other wavelength λ2′. A confocal lens 10a, confocal pinhole 11a, band pass filter 12a and optical detector 13a are disposed on the reflection optical path of the dichroic mirror 8. A confocal lens 10b, confocal pinhole 11b, band pass filter 12b and optical detector 13b are disposed on the reflection optical path of the mirror 9 which is on the transmission optical path of the dichroic mirror 8.

[0023]

A mirror 15 and prism for observation 16 are disposed on a laser optical path scanned by the X-Y scanner 6 via a pupil projection lens 14. Either one of the mirror 15 and prism for observation 16 is disposed on the optical path by a switching apparatus 17. An objective lens 19 is disposed on the reflection optical path of the mirror 15 via an imaging lens 18. Additionally, when the prism for observation 16 is disposed in the optical path, the sample 4 can visually be observed through an eyepiece lens 20.

[0024]

A beam splitter 21 is disposed on the optical path of the laser light extending to the beam splitter 5 from an AOTF 25 in the scanning unit 3. The beam splitter 21 extracts a part of the laser light of two wavelengths of $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm. A prism 22 is disposed on the optical path of the extracted laser light.

[0025]

This prism 22 spectrally resolves the laser light of

two wavelengths λ1 = 488 nm and λ2 = 514.5 nm. That is, the prism 22 splits the laser light into two laser lights of wavelengths λ1 = 488 nm and λ2 = 514.5 nm.

[0026]

A two-split photodiode 23 is disposed in a spectrum emission direction of the prism 22. The two-split photodiode 23 has a function of a light receiving element array for receiving the laser lights spectrally resolved by the prism 22. A split surface of the two-split photodiode 23 is disposed in the same direction as a direction in which spectrum is resolved.

[0027]

A controller 24 inputs a detection signal output from the two-split photodiode 23, and controls the AOTF 25 fixed to an output end of the argon laser 2 based on the detection signal so that respective light intensities of both lines of wavelengths λ1 = 488 nm and λ2 = 514.5 nm become constant.

[0028]

The AOTF 25, under the control of the controller 24, selects the wavelength from two emission wavelengths λ1 = 488 nm and λ2 = 514.5 nm, and continuously controls an emission output.

[0029]

An operation of the scanning type laser microscope constituted as described above will next be described.

[0030]

The laser light of two wavelengths λ1 = 488 nm and

$\lambda 2$ = 514.5 nm is emitted/output from the argon laser 2. The laser light is transmitted through the AOTF 25, and incident upon the beam splitter 21. A part of the laser light is extracted, and incident upon the prism 22.

[0031]

The prism 22 spectrally resolves the laser light into the laser lights of two wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm. Each one of the spectrally resolved laser lights of two wavelengths is incident upon each split surface of the two-split photodiode 23 as the laser light of each line.

[0032]

The two-split photodiode 23 receives each one of the laser lights spectrally resolved by the prism 22 via each split surface and outputs each detection signal.

[0033]

The controller 24 inputs each detection signal output from the two-split photodiode 23, and controls (wavelength selection control, amplitude control) the AOTF 25 fixed to the output end of the argon laser 2 based on the detection signal so that the respective light intensities of both lines of the wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm become constant.

[0034]

In this case, when there are output fluctuations in the lines of laser lights of two wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm emitted/output from the argon laser 2, the fluctuations of the light intensities of these lines are

detected by the two-split photodiode 23, and the AOTF 25 is controlled by the controller 24 so that the respective light intensities of both lines of the wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm become constant.

[0035]

The laser light, controlled so that the respective light intensities of both lines of wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm become constant, is reflected by the beam splitter 5 and scanned in an X-Y direction by the X-Y scanner 6.

[0036]

The scanned laser light is transmitted through the pupil projection lens 14, reflected by the mirror 15, transmitted through the imaging lens 18, forms a spot by the objective lens 19, and is scanned on the sample 4.

[0037]

Respective fluorescent lights of wavelengths $\lambda 1'$ and $\lambda 2'$ emitted from the sample 4 return in a direction opposite to a direction of the optical path. That is, each fluorescent light is passed through the imaging lens 18, mirror 15, pupil projection lens 14, and X-Y scanner 6 from the objective lens 19, further transmitted through the beam splitter 6, reflected by the mirror 7, and incident upon the dichroic mirror 8.

[0038]

In the fluorescent light of two wavelengths $\lambda 1'$, $\lambda 2'$, the dichroic mirror 8 reflects one fluorescent light of

wavelength $\lambda1'$ and transmits the other fluorescent light of wavelength $\lambda2'$. The fluorescent light of the wavelength $\lambda1'$ reflected by the dichroic mirror 8 is passed through the confocal lens 10a, confocal pinhole 11a, and band pass filter 12a and incident upon the optical detector 13a.

[0039]

Additionally, the fluorescent light of the wavelength $\lambda2'$ transmitted through the dichroic mirror 8 is passed through the confocal lens 10b, confocal pinhole 11b, and band pass filter 12b and incident upon the optical detector 13b.

[0040]

These optical detectors 13a, 13b output respective fluorescent intensity signals, and these fluorescent intensity signals are accumulated in synchronization with a drive signal of the X-Y scanner 6, forming images of respective fluorescent lights of two wavelengths $\lambda1'$, $\lambda2'$.

[0041]

As described above, in the first embodiment, the beam splitter 21 extracts a part of the laser light of two wavelengths $\lambda1$ = 488 nm and $\lambda2$ = 514.5 nm, the prism 22 spectrally resolves the laser light of two wavelengths $\lambda1$ = 488 nm and $\lambda2$ = 514.5 nm, and the two-split photodiode 23 detects the intensities of two lines spectrally resolved in this manner. Moreover, the controller 24 controls the AOTF 25 fixed to the output end of the argon laser 2 based on the detection signal output from the two-split photodiode 23 so that the respective light intensities of both lines of

wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm become constant. Thereby, the light intensities of both lines of wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm can simultaneously and steadily be controlled to be constant. Therefore, for example, the cell in which the GFP and YFP are subjected to the gene development can be observed as the sample 4 over a long time with a high reliability. Furthermore, since the two-split photodiode 23 is used, the laser microscope can inexpensively be constituted.

[0042]

(2) A second embodiment of the present invention will next be described with reference to the accompanying drawings. Additionally, the same part as that of FIG. 1 is denoted with the same reference numeral, and detailed description thereof is omitted.

[0043]

FIG. 2 is a constitution diagram of the laser scanning microscope. An argon laser 31, helium neon laser 32, and helium neon laser 33 are disposed in a base 30. The argon laser 31 mainly emits/outputs a laser light of three wavelengths $\lambda 1$ = 488 nm, $\lambda 2$ = 514.5 nm, $\lambda 3$ = 457.9 nm. The helium neon laser 32 emits/outputs the laser light of a wavelength $\lambda 4$ = 543 nm. The helium neon laser 33 emits/ outputs the laser light of a wavelength $\lambda 5$ = 633 nm. For these lasers, a dichroic mirror 34 is disposed on an emission optical path of the argon laser 31, a dichroic mirror 35 is disposed on the emission optical path of the helium neon

laser 32, and a mirror 36 is disposed on the emission optical path of the helium neon laser 33. Therefore, the respective laser lights emitted/output from these lasers 31, 32, 33 are combined into one laser light.

[0044]

Moreover, the AOTF 25 is fixed to the emission end of one laser light in the base 30. The AOTF 25 selects an arbitrary combination of wavelengths with respect to five emission wavelengths $\lambda 1$ = 488 nm, $\lambda 2$ = 514.5 nm, $\lambda 3$ = 457.9 nm, $\lambda 4$ = 543 nm, $\lambda 5$ = 633 nm, and continuously controls the emission output by the control of the controller 24.

[0045]

A fiber coupler 39 is fixed to the emission end of the AOTF 25. One end of an optical fiber 38 is fixed to the fiber coupler 39, and a converging lens 37 is disposed inside the coupler. One optical fiber end 38a of the optical fiber 38 is positioned in a converging position of the converging lens 37 in the fiber coupler 39.

[0046]

On the other hand, one block 40 for resolving and monitoring the spectrum is detachably attached to the scanning unit 3 constituting the laser microscope main body. Other optical fiber end 38b of the optical fiber 38 is inserted/fixed into the block 40 as shown in an enlarged constitution diagram of FIG. 3. A beam splitter 42 is disposed via a collimator lens 41 on the optical path of the laser light emitted from the optical fiber end 38b. The beam

splitter 42 extracts a part of the laser light collimated by the collimator lens 41.

[0047]

A prism 43 is disposed on the optical path of the laser light extracted by the beam splitter 42.

[0048]

The prism 43 spectrally resolves the laser light emitted from the optical fiber end 38b into the laser lights of five wavelengths $\lambda1$ = 488 nm, $\lambda2$ = 514.5 nm, $\lambda3$ = 457.9 nm, $\lambda4$ = 543 nm, $\lambda5$ = 633 nm.

[0049]

A one-dimensional CCD 45 is disposed via a converging lens 44 in a spectrum emission direction of the prism 43. The one-dimensional CCD 45 functions as a light receiving element array that receives the laser lights spectrally resolved by the prism 45. The one-dimensional CCD 45 has five divided block surfaces corresponding to the respective lines of the wavelengths $\lambda1$ = 488 nm, $\lambda2$ = 514.5 nm, $\lambda3$ = 457.9 nm, $\lambda4$ = 543 nm, $\lambda5$ = 633 nm, and outputs each detection signal via each block surface. Additionally, the detection signals indicate a sum of output signals of the respective elements for the respective block surfaces.

[0050]

The controller 24 has a function of inputting the respective detection signals output from the one-dimensional CCD 45, and controlling the AOTF 25 based on the detection signals so that the respective light intensities of the

respective lines of the wavelengths λ1 = 488 nm, λ2 = 514.5 nm, λ3 = 457.9 nm, λ4 = 543 nm, λ5 = 633 nm become constant.

[0051]

Additionally, dichroic mirrors 8a, 8b, 8c, confocal lenses 10a, 10b, 10c, 10d, confocal pinholes 11a, 11b, 11c, 11d, band pass filters 12a, 12b, 12c, 12d, optical detectors 13a, 13b, 13c, 13d, and mirror 9 are disposed in the scanning unit 3, so that the sample 4 marked with four types of fluorescent lights at the maximum can simultaneously be observed.

[0052]

The operation of the laser scanning microscope constituted as described above will next be described.

[0053]

The argon laser 31 emits/outputs the laser light of three wavelengths λ1 = 488 nm, λ2 = 514.5 nm, λ3 = 457.9 nm. The helium neon laser 32 emits/outputs the laser light of wavelength λ4 = 543 nm. The helium neon laser 33 emits/outputs the laser light of wavelength λ5 = 633 nm. These laser lights are combined into one laser light by the dichroic mirrors 34, 35 and mirror 36.

[0054]

The laser light having the wavelength selected in the arbitrary combination of wavelengths from the combined laser light passes the AOTF 25. The laser light is converged by the converging lens 37 and incident upon the one optical

fiber end 38a of the optical fiber 38. The laser light is propagated through the optical fiber 38 and emitted from the other optical fiber end 38b inserted into the block 40.

[0055]

The laser light emitted from the optical fiber end 38b is collimated by the collimator lens 41, and a part of the laser light is extracted by the beam splitter 42 and incident upon the prism 43.

[0056]

The prism 43 spectrally resolves the laser light emitted from the optical fiber end 38b into the laser lights of five wavelengths $\lambda 1$ = 488 nm, $\lambda 2$ = 514.5 nm, $\lambda 3$ = 457.9 nm, $\lambda 4$ = 543 nm, $\lambda 5$ = 633 nm. The laser lights of respective lines of wavelengths $\lambda 1$ = 488 nm, $\lambda 2$ = 514.5 nm, $\lambda 3$ = 457.9 nm, $\lambda 4$ = 543 nm, $\lambda 5$ = 633 nm spectrally resolved in this manner are incident upon five block surfaces of the one-dimensional CCD 45.

[0057]

The one-dimensional CCD 45 receives the laser lights spectrally resolved by the prism 45, and outputs each detection signal via each block surface.

[0058]

The controller 24 inputs the respective detection signals output from the one-dimensional CCD 45, and controls the AOTF 25 based on the detection signals so that the respective light intensities of respective lines of wavelengths $\lambda 1$ = 488 nm, $\lambda 2$ = 514.5 nm, $\lambda 3$ = 457.9 nm,

$\lambda 4$ = 543 nm, $\lambda 5$ = 633 nm become constant.

[0059]

In this case, when there is the output fluctuation in the laser light of the wavelength oscillated in the arbitrary combination of the respective lines of wavelengths $\lambda 1$ = 488 nm, $\lambda 2$ = 514.5 nm, $\lambda 3$ = 457.9 nm, $\lambda 4$ = 543 nm, $\lambda 5$ = 633 nm, the one-dimensional CCD 45 detects the fluctuations of the light intensities of these lines and controls the AOTF 25 so that the respective light intensities of respective lines of wavelengths selected among $\lambda 1$ = 488 nm, $\lambda 2$ = 514.5 nm, $\lambda 3$ = 457.9 nm, $\lambda 4$ = 543 nm, $\lambda 5$ = 633 nm become constant.

[0060]

The laser light, controlled so that the respective light intensities of lines of wavelengths become constant, is reflected by the beam splitter 5 and scanned in the X-Y direction by the X-Y scanner 6.

[0061]

The scanned laser light is transmitted through the pupil projection lens 14, reflected by the mirror 15, transmitted through the imaging lens 18, forms the spot by the objective lens 19, and is scanned on the sample 4.

[0062]

Each fluorescent light emitted from the sample 4 returns in the direction opposite to the direction of the optical path. That is, each fluorescent light is passed through the imaging lens 18, mirror 15, pupil projection lens

14, and X-Y scanner 6 from the objective lens 19, further transmitted through the beam splitter 5, reflected by the mirror 7, and incident upon the dichroic mirror 8a. Then, by each of the dichroic mirrors 8a to 8c, the fluorescent lights of respective wavelengths are divided and are incident upon each of the optical detectors 13a, 13b, 13c, 13d. Thus, when respective fluorescent intensity signals output from these optical detectors are input and accumulated in synchronization with the drive signal of the X-Y scanner 6, forming images, and the respective fluorescent images of the sample 15 marked with four types of fluorescent lights at the maximum are formed.

[0063]

As described above, in the second embodiment, the beam splitter 42 extracts a part of the laser light of the arbitrary combination of wavelengths selected from the laser light of five wavelengths λ1 = 488 nm, λ2 = 514.5 nm, λ3 = 457.9 nm, λ4 = 543 nm, λ5 = 633 nm, the prism 43 spectrally resolves the laser light of these wavelengths, and the one-dimensional CCD 45 detects the respective intensities of the lines spectrally resolved in this manner. Moreover, the controller 24 controls the AOTF 25 based on the respective detection signals output from the one-dimensional CCD 45 so that the respective light intensities of the lines become constant. Therefore, the light intensities of five lines of wavelengths λ1 = 488 nm, λ2 = 514.5 nm, λ3 = 457.9 nm, λ4 = 543 nm, λ5 = 633 nm can simultaneously and steadily be

controlled to be constant. As a result, the gene developed cell of the sample 4 can be observed for a long time with a high reliability. Moreover, since the one-dimensional CCD 45 is used, a plurality of laser lined can be dealt with, and a design with a high degree of freedom is possible.

[0064]

Furthermore, the collimator lens 41, beam splitter 42, prism 43, converging lens 44 and one-dimensional CCD 45 are constituted as one block 40. For example, there is another laser scanning microscope in which the scanning unit 3 is connected to the inverted type microscope main body. In this case, when erected and inverted types are to be selectively used in accordance with a purpose of use, a block including a laser light source and CCD for detecting the light intensity of each line can be used in common, and can inexpensively be constituted.

[0065]

The present invention is not limited to the first and second embodiments described above and can be practiced in various modifications without departing from the spirit and scope thereof.

[0066]

Further, the embodiments include various phases of the invention, and thus, various inventions can be extracted in accordance with proper combinations of a plurality of disclosed components. For example, even if some components are excluded from the entire components represented in the

embodiments, as far as the requirement described in "Object of the Invention" is solved and the effect described in "Advantage of the Invention" is attained, the remaining components can be practiced as the invention.

[0067]

For example, the spectral resolution means in the first and second embodiments is not limited to the prisms 22, 43, and a diffraction grating or a beam splitter may also be used.

[0068]

[Advantage of the Invention]

As has been described in detail, the present invention can provide a laser microscope capable of simultaneously and steadily controlling an intensity of a laser light constituted of a plurality of wavelengths with which a sample is to be irradiated for each wavelength.

[Brief Description of the Drawings]

[FIG. 1]

A constitution diagram showing a first embodiment of a scanning type laser microscope according to the present invention.

[FIG. 2]

A constitution diagram showing a second embodiment of the scanning type laser microscope according to the present invention.

[FIG. 3]

An enlarged constitution diagram in a block in the

second embodiment of the scanning type laser microscope according to the present invention.

[Explanation of Reference Symbols]

1: Base

2: Argon laser

3: Scanning unit

4: Sample

5: Beam splitter

6: X-Y scanner

7: Mirror

8: Dichroic mirror

9: Mirror

10a, 10b: Confocal lens

11a, 11b: Confocal pinhole

12a, 12b: Band pass filter

13a, 13b: Optical detector

14: Pupil projection lens

15: Mirror

16: Prism for observation

17: Switching apparatus

18: Imaging lens

19: Objective lens

20: Eyepiece lens

21: Beam splitter

22: Prism

23: Two-split photodiode

24: Controller

25: AOTF

30: Base

31: Argon laser

32, 33: Helium neon laser

34, 35: Dichroic mirror

36: Mirror

37: Converging lens

38: Optical fiber

39: Fiber coupler

40: Block

41: Collimator lens

42: Beam splitter

43: Prism

44: Converging lens

45: One-dimensional CCD

APPLICANT'S PAST DATA

Identification Number [000000376]

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[Reason for Change] New Registration

[Address] 43-2, Hatagaya 2-chome,
Shibuya-ku, Tokyo

[Name] OLYMPUS OPTICAL CO., LTD.

[Document] ABSTRACT

[Abstract]

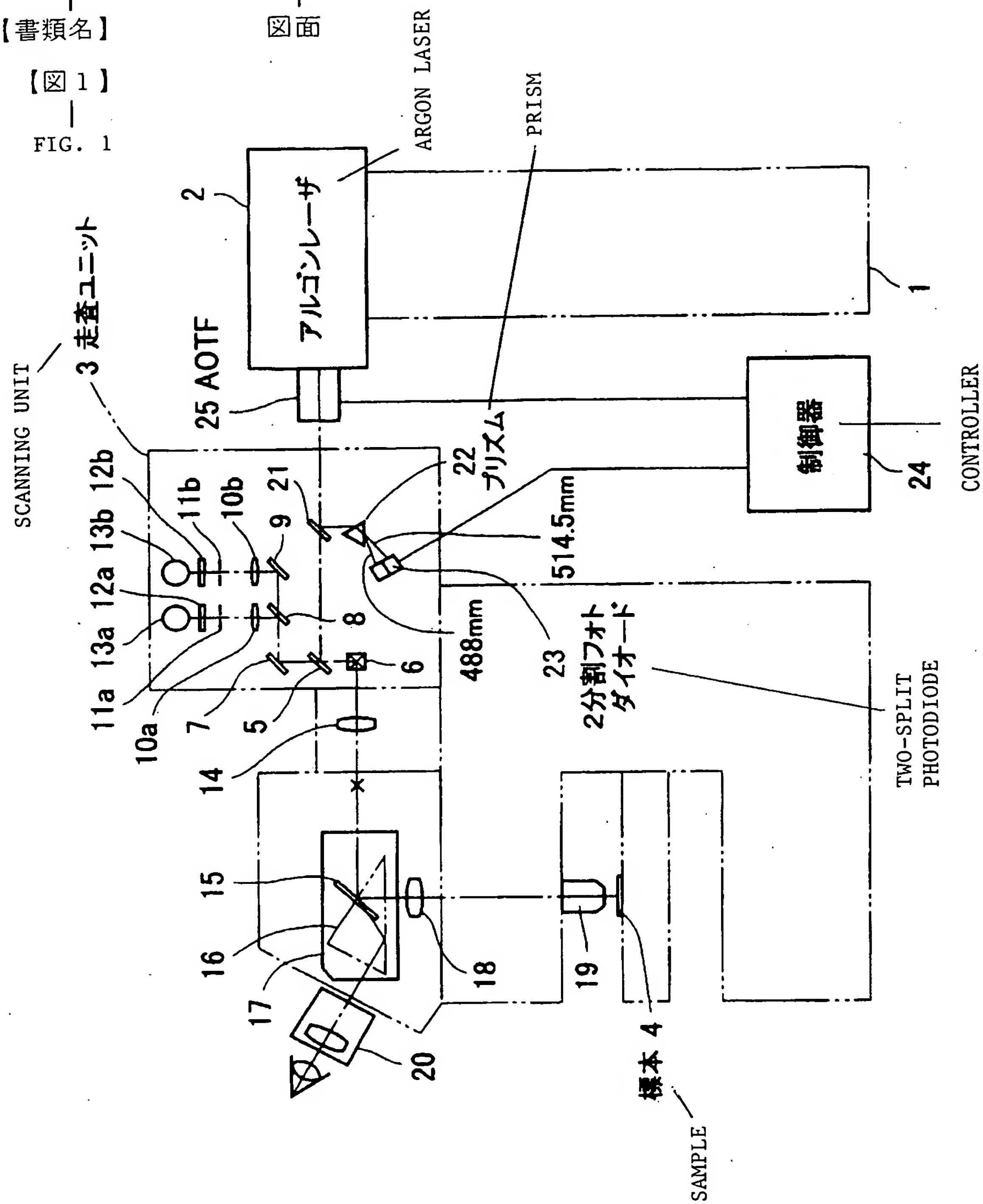
[Object] To simultaneously and steadily control an intensity of a laser light constituted of a plurality of wavelengths with which a sample is to be irradiated for each wavelength.

[Means for Achieving the Object] A beam splitter 21 extracts a part of a laser light of two wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm, a prism 22 spectrally resolves the laser light of the two wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm, a two-split photodiode 23 detects intensities of two lines spectrally resolved in this manner, and a controller 24 controls an AOTF 25 fixed to an output end of an argon laser 2 based on a detection signal output from the two-split photodiode 23 so that respective light intensities of both lines of wavelengths $\lambda 1$ = 488 nm and $\lambda 2$ = 514.5 nm become constant.

[Elected Figure] FIG. 1

NAME OF DOCUMENT 【書類名】 DRAWINGS 図面

【図1】
FIG. 1



【図2】
FIG. 2

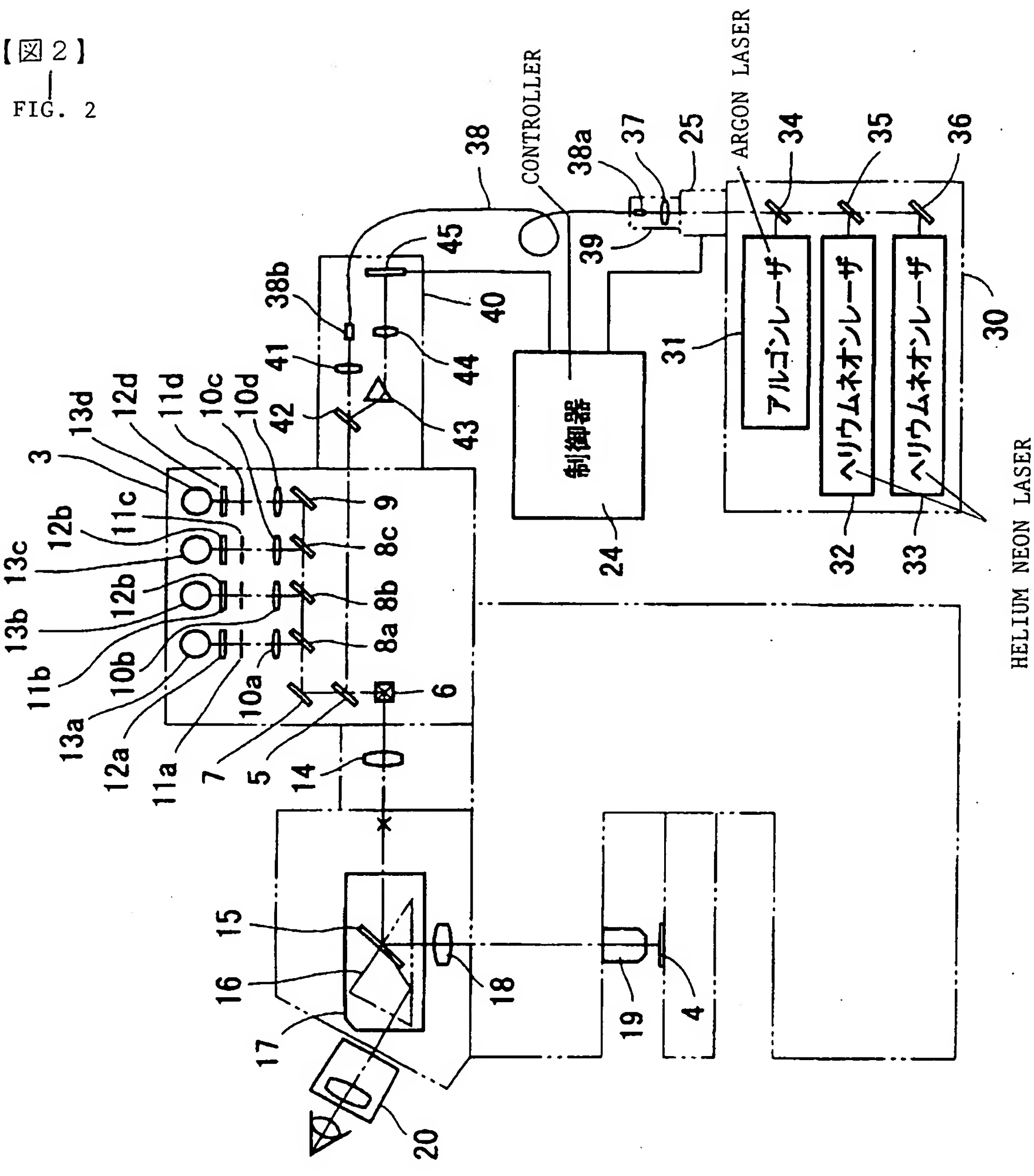
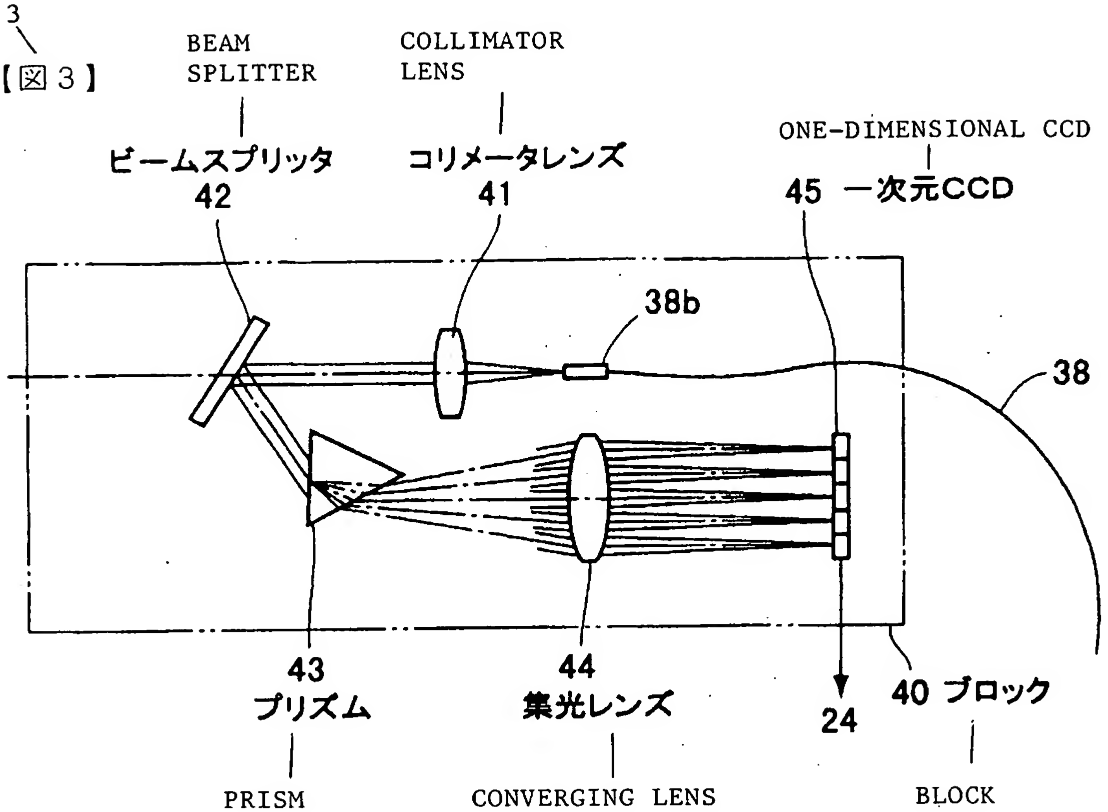

CONTROLLER
ARGON LASER
制御器
アルゴンレーザ
ヘリウムネオンレーザ
ヘリウムネオンレーザ
HELIUM NEON LASER

FIG. 3

【図３】


BEAM SPLITTER
ビームスプリッタ
42
COLLIMATOR LENS
コリメータレンズ
41
ONE-DIMENSIONAL CCD
45 一次元CCD
38b
38
43
プリズム
PRISM
44
集光レンズ
CONVERGING LENS
24
40 ブロック
BLOCK